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Short communication

Phototoxicity of fluoranthene to two freshwater crustaceans, *Hyalella azteca* and *Daphnia magna*: measures of feeding inhibition as a toxicological endpoint

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Abstract

We investigated the effects of fluoranthene, a phototoxic polycyclic aromatic hydrocarbon (PAH), on the feeding inhibition of two aquatic crustaceans, the cladoceran *Daphnia magna* and the amphipod *Hyalella azteca*. We used two measures of feeding inhibition: beta-galactosidase enzyme activity levels in *D. magna* and *H. azteca*, and leaf disc processing in *H. azteca*. We also investigated the survival of *D. magna* in a 48 h toxicity assay, and the survival and growth of *H. azteca* over the 10 d leaf disc processing experiment. In UV-fluoranthene treatments, survival of *H. azteca* was greater than expected based on the results of previous experiments. Leaf disc processing of *H. azteca* was significantly reduced at 25 μg fluoranthene/L under UV light. Growth of *H. azteca* was not significantly affected in any treatments. In UV-fluoranthene treatments, beta-galactosidase activity levels of both *D. magna* and *H. azteca* were reduced. However, the response of *H. azteca* to the UV-fluoranthene treatments in the enzyme assay was more similar to the results of a standard toxicity test, than was the response of *D. magna* in the enzyme assay.

Introduction

Pollution of freshwater ecosystems from sources such as oil spills, fossil fuel combustion and urban runoff often results in contamination of the water with polycyclic aromatic hydrocarbons (PAHs) (Hoffman et al., 1984; Oris & Giesy, 1986; Newsted & Giesy, 1987; Arfsten et al., 1996; Ireland et al., 1996). Some PAHs are acutely toxic to freshwater biota in the presence of ultraviolet (UV) light (Oris & Giesy, 1986; Newsted & Giesy, 1987; Oris et al., 1990; Hall & Oris, 1991; McCloskey & Oris, 1993; Arfsten et al., 1996; Ireland et al., 1996; Weinstein et al., 1997). Exposure to low (μg/L) aqueous levels of anthracene with UV light caused lethal effects in daphnia (*Daphnia magna*), fathead minnows (*Pimephales promelas*) and bluegill sunfish (*Lepomis macrochirus*) (Oris & Giesy, 1986; Oris et al., 1990). In aquatic fish and invertebrates, this lethality is likely due to disruption of cellular membrane function (McCloskey & Oris, 1993; Weinstein et al., 1997).

In aquatic biota, sublethal physiological effects due to phototoxic PAHs may also occur. For example, phototoxic PAHs impaired cladoceran reproduction (Ireland et al., 1996; Arfsten et al., 1996). Phototoxic PAHs may also contribute to detrimental effects such as reduced growth and reduced food intake. Several studies demonstrate the application of measuring feeding behavior as a toxicological endpoint to evaluate the impact of other freshwater pollutants (Naylor et al., 1989; Crane & Maltby, 1991; Taylor et al., 1993; Maltby & Crane, 1994; Maltby et al., 1995;

Malbouisson et al., 1995; Blockwell et al., 1998). Recently, several surrogate measures of feeding inhibition have been developed as rapid-response toxicity tests. These tests typically measure ingestion rate or enzyme activity of aquatic invertebrates using a labeled or fluorescent substrate (Hayes et al., 1993a; Janssen et al., 1993; Juchelka & Snell, 1995; Hayes et al., 1996; Bitton et al., 1996). For example, the IQ Toxicity Test™ developed by AquaSurvey, Inc. measures beta-galactosidase activity as an estimate of feeding inhibition in aquatic invertebrates (Hayes et al., 1993a; Hayes et al., 1996).

In this study, we investigated the effects of fluoranthene, a phototoxic PAH, on the feeding inhibition of two aquatic invertebrates, the cladoceran *Daphnia magna* and the amphipod *Hyalella azteca*. We used enzyme inhibition of *D. magna* and *H. azteca* and leaf disc processing of *H. azteca* as measures of feeding inhibition. We also investigated the survival of *D. magna* in a standard 48 h toxicity assay, and the survival and growth of *H. azteca* over the 10 d leaf disc processing experiment.

Methods and materials

Test organisms and experimental design

H. azteca and *D. magna* were cultured in the laboratory following U.S. EPA procedures (U.S. EPA 1994). *H. azteca* were 7–14 days old at the beginning of the 10 d experiments and 14–21 d old at the beginning of the IQ Toxicity Test™. *D. magna* were 24–36 h old at the beginning of experiments. Dry weight of *H. azteca* was used to measure growth. Organisms were dried in an oven at 100 °C for 24 h and then weighed to the nearest 0.01 mg (U.S. EPA, 1994). A subsample of 30 organisms from the pool of experimental animals was measured at test initiation, and surviving organisms were measured at test completion. The change in weight was recorded as growth.

The 10 d leaf disc processing experiment was conducted using one leaf disc per individual *H. azteca*, with 30 replicates per treatment group. Treatment groups were: 0, 6.25, 12.5 and 25 μg fluoranthene/L. An additional leaf-only control group accounted for any changes in leaf weight that were not due to the action of test organisms. Following guidelines of AquaSurvey Inc., the IQ Toxicity Test™ consisted of three replicates per treatment with six individuals per replicate. A standard 48 h *D. magna* toxicity test was also conducted using three replicates per treatment with 10 individuals per replicate. Treatment groups in the 48 h test were: 0, 6.25, 12.5, 25, 50 and 100 μg fluoranthene/L. Water in treatments was renewed daily.

In all experiments, the fluoranthene treatment series was duplicated with and without UV light. Thus, all experiments included control treatments for the effects of fluoranthene alone without UV, and of UV alone without fluoranthene.

Leaf disc preparation

The change in weight of a maple leaf disc was used as an endpoint to assess the detritivorous activity of *H. azteca* (Naylor et al., 1989). Red maple (*Acer rubrum*) leaves were collected shortly after abscission. To increase palatability, leaves were conditioned with *Cladosporium* fungus for approximately 3 months before use (Naylor et al., 1989; Taylor et al., 1993). Using a cork borer, leaves were then cut into 1 cm diameter discs for testing. Leaf discs were dried in an oven at 60 °C for 48 h prior to initial and final weighing to the nearest 0.01 mg (Naylor et al., 1989).

IQ Toxicity Test™

H. azteca were starved for 6 h, exposed to test conditions for one hour, and incubated with substrate (0.4 mg/ml 2-methylumbelliferone) for 20 minutes (Hayes et al., 1993b). *D. magna* were starved for 6 h, exposed to test conditions for one hour, and incubated with substrate (0.2 mg/ml 2-methylumbelliferone) for 15 minutes (Hayes et al., 1993a).

Water quality

Spring water diluted with Nanopure® water to a hardness of 160–175 mg $CaCO_3$/L was used in culturing and experiments. During the experiment, dissolved oxygen (D.O.) and temperature were recorded daily. Conductivity, pH, alkalinity and hardness were measured at the initiation and completion of the experiment. D.O. was measured with a YSI Model 57 probe, and conductivity was measured with a YSI Model 335-C-T probe. Alkalinity was measured by titration with sulfuric acid, and hardness was measured by titration with EDTA (APHA, 1995).

Laboratory lighting

To simulate UV light, two fluorescent bulbs, one UV-A bulb and one UV-B bulb, were used. UV light bulbs (40 watt size; Wesco Distributors, Dayton, OH, U.S.A.) were placed alongside fluorescent light bulbs. The UV-A bulb had a spectral output ranging from 310 to 420 nm, peaking at 350 nm. The UV-B bulb had a spectral output ranging from 250 to 400 nm, peaking at 290 nm. An acetate screen was used to attenuate wavelengths below 290 nm. During the experiments, UV-A and UV-B intensity were measured daily using a Macam Model 103 radiometer (Livingstone, Scotland, U.K.). UV-A intensity ranged from 60 to 72 μW/cm^2 and UV-B intensity ranged from 2 to 5 μW/cm^2. No-UV treatments were conducted under fluorescent lighting only. Levels of UV in the No-UV treatments were negligible (less than 1 μW/cm^2 UV-A and less than 0.01 μW/cm^2 UV-B).

Test compounds and concentrations

Aqueous stock solutions of fluoranthene (Aldrich Chemical Co., MW 202.26; 98% purity) were made following the method of shell-coating described in Newsted & Giesy (1987). A glass flask was coated with a known amount of fluoranthene dissolved in HPLC grade acetone, and the acetone was allowed to evaporate overnight. The following day, Nanopure® water of a known volume was added and the flask was allowed to equilibrate over a period of 5 days (Newsted & Giesy, 1987). Thus stock solutions of fluoranthene well below levels of aqueous solubility (see Mackay & Shiu, 1977) were prepared and diluted for experiments.

Chemical analysis

The actual concentrations of fluoranthene in experimental treatments were measured using gas chromatography with flame ionization detection (GC-FID) (Carlo-Erba 6000 Vega Series; Italy) (Ireland et al., 1996). Fluoranthene was first extracted from water samples (250 ml) using packed C-18 columns (J.T. Baker, Paris, KY, U.S.A.). The column was eluted with a known amount of HPLC grade methanol. The methanol extract was then injected into the GC-FID using a splitless injection technique and a nonpolar SE®-54 column (Ireland et al., 1996). Linear regression with a calibration curve of 5 standard concentrations was used for quantification of fluoranthene. Fluoranthene concentrations were determined from high and low experimental treatment samples at the beginning and at the completion of an experiment. Each sample was injected two times, and the average peak ratio of the duplicate injections was used in calculating the concentration.

Statistics

In the 10 d *H. azteca* experiment, statistically significant effects among the treatment groups were determined via ANOVA followed by the Wilcoxon Rank-Sum Test using the software program Toxstat version 3.0 (Laramie, Wyoming, U.S.A.). For the 48 h toxicity test and the IQ Toxicity Test™, Probit analysis calculated IC50s and their associated confidence limits (Probit version 1.5 and Trimmed Spearman Karber version 1.5, U.S. EPA, Cincinnati, OH, U.S.A.).

Results

Water quality

Dissolved oxygen ranged from 6.8 to 8.0 mg/L in our experiments; temperature, 22 to 24 °C; pH, 8.01 to 8.09; alkalinity, 100 to 120 mg $CaCO_3$/L; hardness, 158 to 198 mg $CaCO_3$/L; conductivity, 280 to 395 μmhos/cm.

Chemical analysis

Water samples extracted using C-18 packed columns revealed 80 to 100% recovery of the surrogate standard. Blank samples indicated no compounds present that would interfere with fluoranthene measurement. The nominal 25 μg/L treatment was measured at 24.2 μg/L at the beginning of the leaf disc processing experiment and 15.7 μg/L at the completion of the experiment. The 6.25 μg/L treatment was measured at 6.1 μg/L at the beginning of the leaf disc processing experiment and 4.2 μg/L at the completion of the experiment. For the IQ Toxicity Test™ and the *D. magna* standard toxicity test, the 100 μg/L treatment was measured at 101.3 μg/L at the beginning of the test and 87 μg/L at the completion of the 48 h *D. magna* standard toxicity test. The 25 μg/L treatment in the IQ Toxicity Test™ and the 48 h *D. magna* test was measured at 27.3 μg/L at the beginning of the test and 17.7 μg/L at the completion of the 48 h *D. magna* toxicity test.

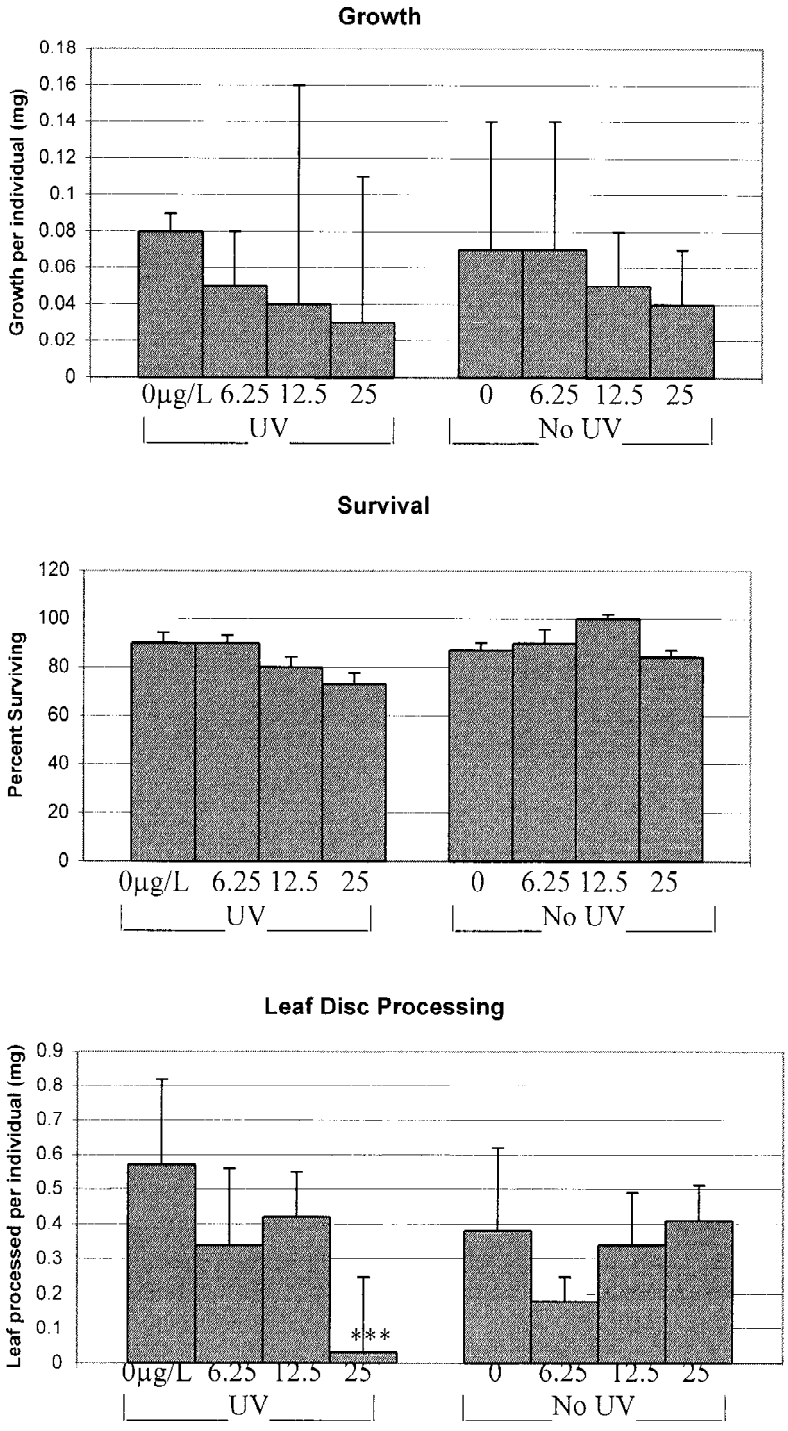

Figure 1. Growth, survival and leaf disc processing of *Hyalella azteca* exposed to fluoranthene and UV light. Treatments are in the units 'μg fluoranthene/L'. Error bars represent the standard deviation. ***Indicates treatments that are statistically significantly different from the control treatment.

Leaf disc processing experiment

Figure 1 illustrates the response of *H. azteca* as exhibited by leaf disc processing, growth and survival. Leaf disc processing was significantly reduced in the 25 μg/L treatment with UV. Growth was not statistically significantly affected under any treatment regime, although there was a non-significant trend toward decreased growth in the UV/fluoranthene treatments. Survival was not statistically significantly affected under any treatment regime.

IQ Toxicity Test^{TM} and 48 h *D. magna* toxicity test

The IC-50 for fluoranthene under UV light to *H. azteca* was 10.9 μg/L (95% confidence interval: 1.1–18.9 μg/L). The 48 h LC50 of photo-induced toxicity of fluoranthene to *D. magna* was 8.7 (8.0–9.5) μg/L, while the IC50 determined by the IQ Toxicity Test^{TM} was 30.1 (22.9–39.6) μg/L. No significant effects were found in the no-UV treatments in the IQ Toxicity Test^{TM} or in the *D. magna* 48 h test.

Discussion and conclusions

In UV/fluoranthene treatments with leaf discs, survival of *H. azteca* was greater than expected based on previous experiments in our laboratory. Previous work, without leaf discs, indicated a 10 d LC-50 for *H. azteca* of approximately 7.3 μg fluoranthene/L (95% confidence interval, 5.5–8.7 μg fluoranthene/L) (Hatch & Burton, 1999). The greater survival in our leaf disc exposures could be due to *H. azteca* avoiding UV exposure under the refuge of the leaf discs. Other research has suggested that photo-induced toxicity of PAHs requires the simultaneous exposure of organisms to UV and PAH (Oris & Giesy, 1986; Newsted & Giesy, 1987; Oris et al., 1990). Observations of behavior suggest that *H. azteca* spent more time burrowing under leaves in UV/fluoranthene treatments than in no-UV treatments or in UV treatments without fluoranthene (Hatch & Burton, 1999).

Although we did not observe lethality of *H. azteca* in UV-fluoranthene treatments with leaf discs, leaf disc processing in the 25 μg/L treatment under UV light was significantly reduced, suggesting a sublethal effect. Other work shows that food intake is a particularly sensitive aspect of an organism's scope-for-growth (Naylor et al., 1989) and a sensitive ecotoxicological endpoint (Malbouisson et al., 1995). Growth of *H. azteca* was not significantly impaired in any treatment and was comparable to growth observed in standard 10 d toxicity exposures.

The IQ Toxicity Test^{TM} revealed that both test organisms were sensitive to phototoxic effects of fluoranthene. The IC50 for enzyme inhibition obtained via the IQ Toxicity Test^{TM} indicated that the sensitivity of *H. azteca* to this test (IC50, approximately 10.9 μg/L) was similar to the sensitivity of *H. azteca* in a typical 10 d toxicity test (LC50, approximately 7.3 μg/L). However, the IC50 for *D. magna* (approximately 30.1 μg/L) suggested greater tolerance to phototoxic effects than the results of the 48 h *D. magna* exposure (LC50, approximately 8.7 μg/L). Possibly, *D. magna* were slower to uptake fluoranthene than *H. azteca* and so did not demonstrate effects in the one-hour exposure.

For both *D. magna* and *H. azteca*, the confidence limits for the IC-50 were broader than the confidence limits calculated for the LC-50. One possible reason for the greater variability in the IQ Toxicity Test^{TM} could be the smaller sample size that was used (6 individuals per replicate) in comparison to the standard toxicity tests (10 individuals per replicate). Overall, the IQ Toxicity Test^{TM} was useful as an approximation of the range at which phototoxic effects occurred.

Aquatic animals can repair some of the damage caused by photo-toxic PAHs (Oris & Giesy, 1986). If energy is spent repairing cellular damage caused by this photo-toxic stress, less energy may be available to the organism for other processes such as feeding. A general reduction in feeding due to toxic stress has been documented in laboratory and field studies (Crane & Maltby, 1991; Taylor et al., 1993; Blockwell et al., 1998). In this study, we suggest that the stress of exposure to phototoxic fluoranthene reduced the feeding efficiency of the two freshwater crustaceans, *H. azteca* and *D. magna*.

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